The Role of Base Excision Repair in generating mutations from aromatic hydrocarbon- & estrogen-induced DNA damage

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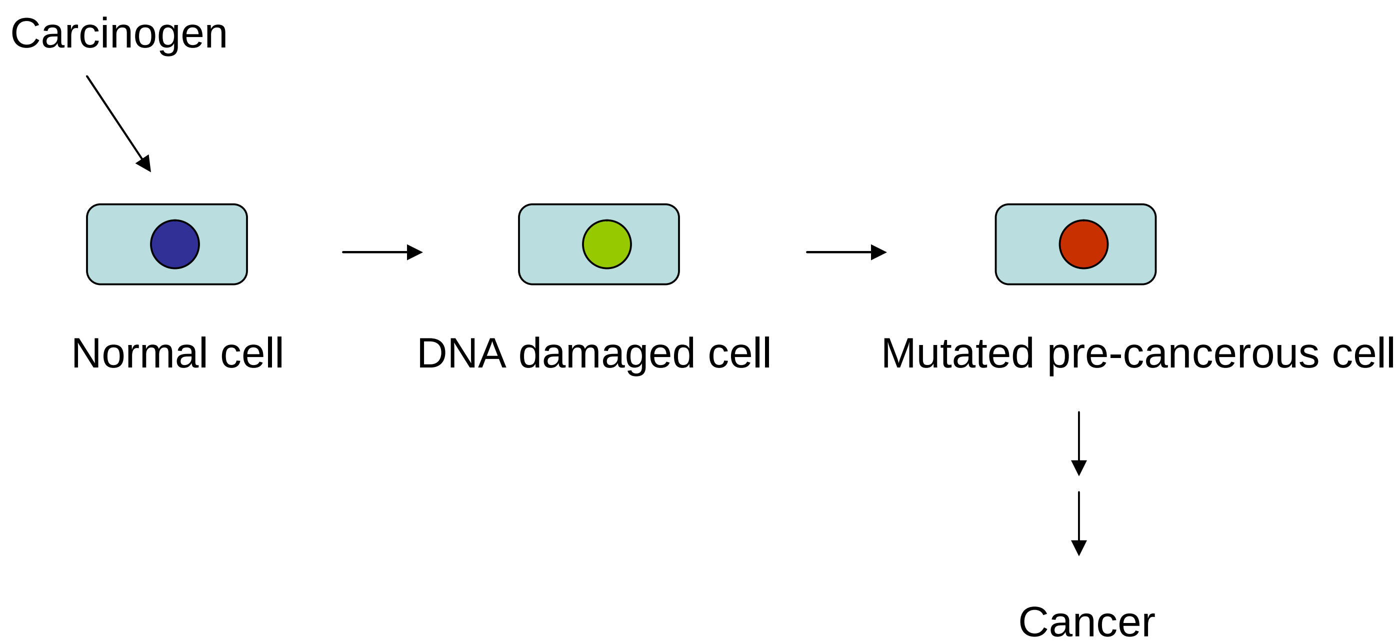
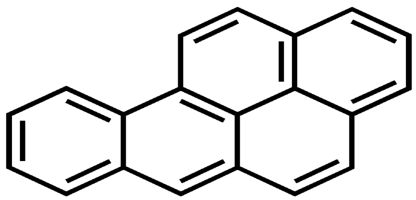
Contents…

Somatic Mutations in Cancer



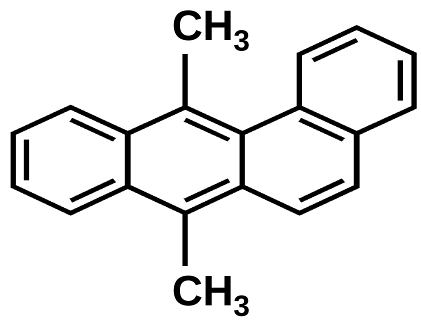
The Carcinogens



Benzo[*a*]pyrene

RC, DE

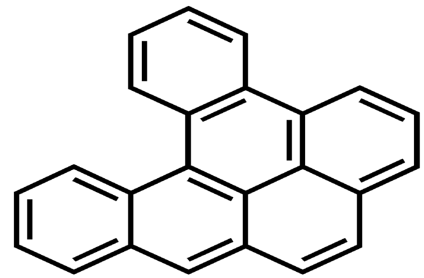
Depur, Stable adducts
71% 29%



7,12-dimethylbenz[*a*]anthracene

RC, DE

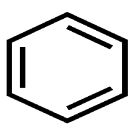
Depur, Stable adducts
99% 1%



Dibenzo[*a,l*]pyrene

RC, DE

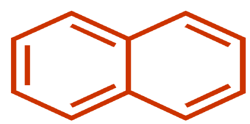
Depur, Stable adducts
99% 1%



Benzene

***o*-Q**

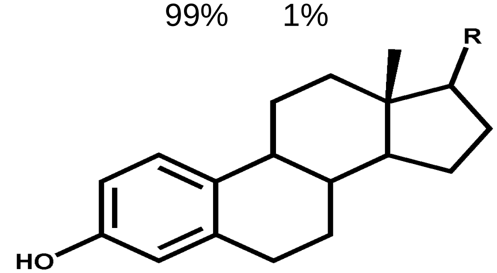
Depur, Stable adducts
99.9%



Naphthalene

***o*-Q**

Depur, Stable adducts
50% 50%

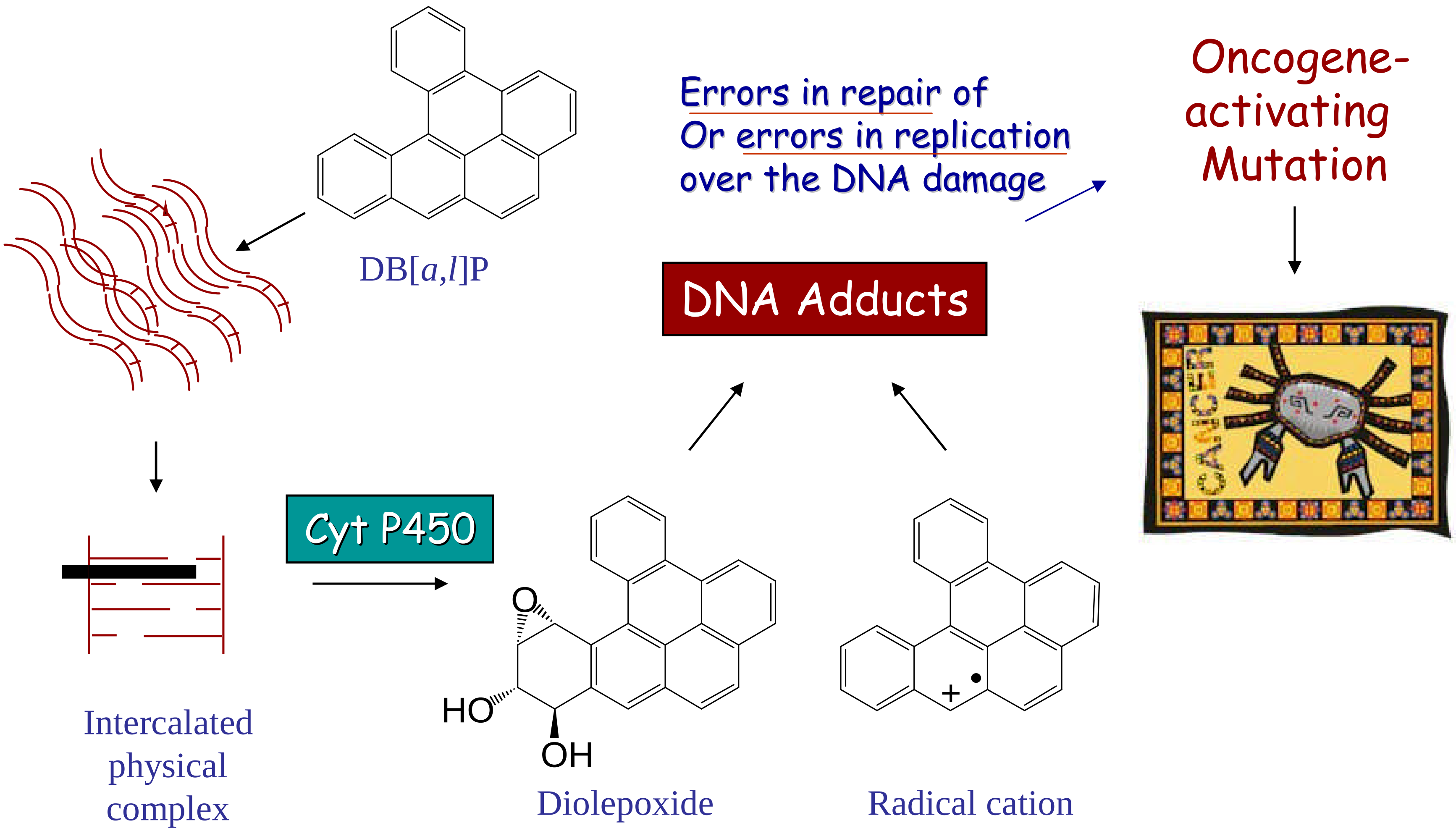


Estrone/Estradiol

***o*-Q**

Depur, Stable adducts
99.9% <0.1%

Carcinogenesis by PAH



Cavalieri and Rogan (1998) Mechanism of tumor initiation by polycyclic aromatic hydrocarbons in mammals. In: Handbook of environmental chemistry. (A.H. Neilson, ed.) Springer-Verlag.

Induction of *ras* mutations is a tumor-initiating event

- Many cancers (including lung[1] and skin[2] cancer) show *ras* gene mutations
- These mutations are formed in the early pre-neoplastic phase[3]
- 'Activating' *ras* mutations can transform cells[4]
- Various animal models (*e.g.* SENCAR mouse skin) have been developed to study the induction of *ras* mutations and their roles in cancer

1. Rodenhuis (1992) Semin Cancer Biol. 3: 241.
2.Vathen der Schroeff et al (1990) J. Invest Dermatol. 94: 423
3. Kemp et al (1994) CSH Symp Quant Biol. 427.
4. Kanda et al (1993) Carcinogenesis 14: 1061-3.

How are the mutations induced? Biological evidence

- Carcinogen exposure blocks replication and induces excision repair.
  - Nucleotide excision repair (NER) removes stable adducts
  - Base excision repair (BER) removes abasic sites formed by the depurinating adducts
- After repair, cells can resume replication and cell division
  - Therefore, by knowing when preneoplastic mutations are formed, we can understand the mutagenic mechanism

Mutagenesis by BP

- BP forms mostly depurinating adducts (71%), but it also forms significant amounts of stable adducts (29%).
- It induces both BER and NER
- Depurinating adducts show a strong relationship with H-*ras* mutations in BP-induced papillomas
- On the other hand, NER-defective mice show increased mutagenesis and tumor formation, suggesting a role of BP-stable adducts

Braithwaite et al (1998) Carcinogenesis 19: 1239
deVries et al (1997) Mol Carcinog 19: 46: Carcinogenesis 18: 2327
Wijnhoven et al (2000) Cancer Res 60: 5681
Chakravarti et al (1995) PNAS 92: 10422

BPDE-adducted DNA forms tumorigenic mutations in proliferating cells

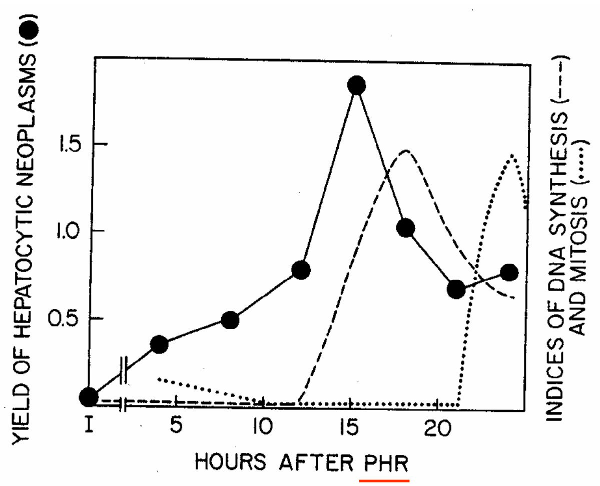
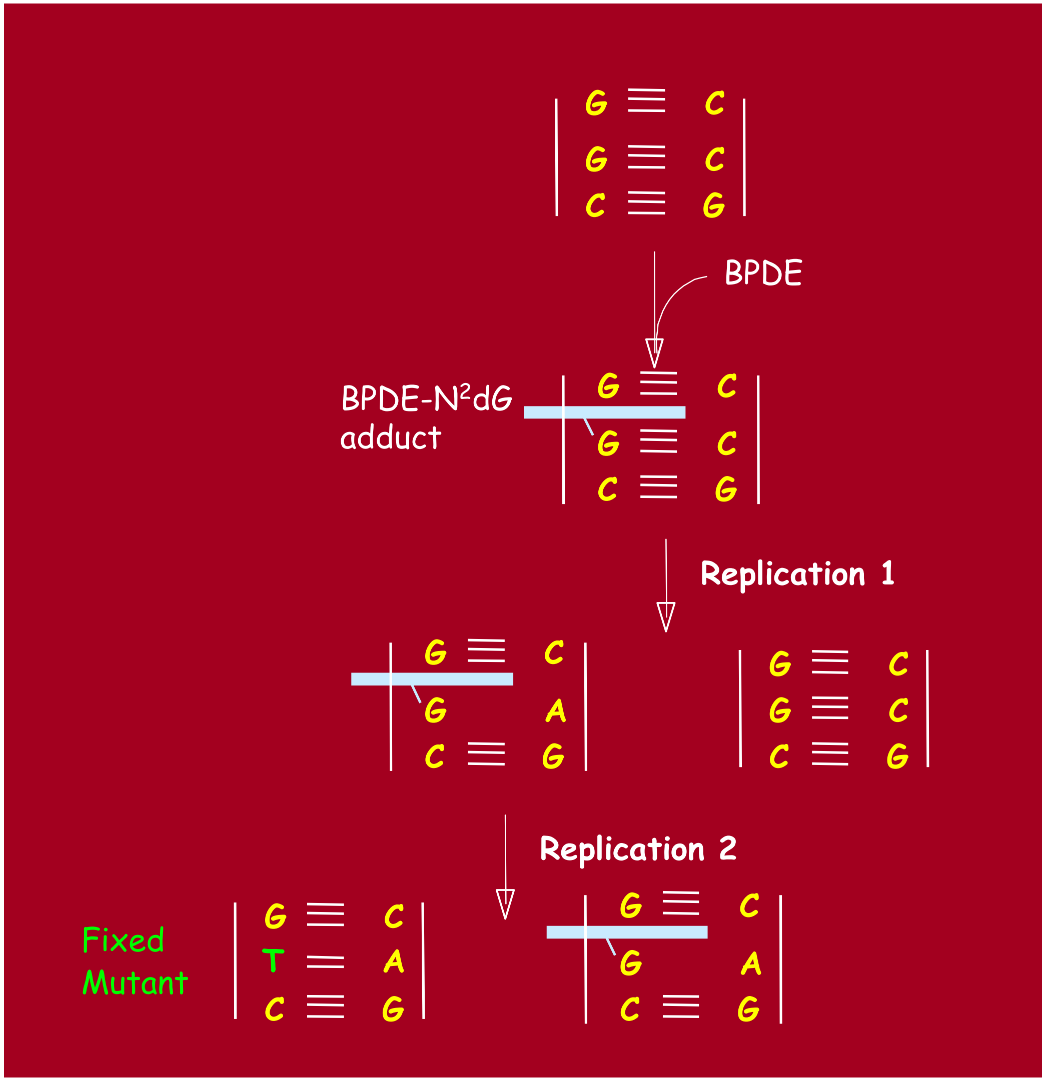
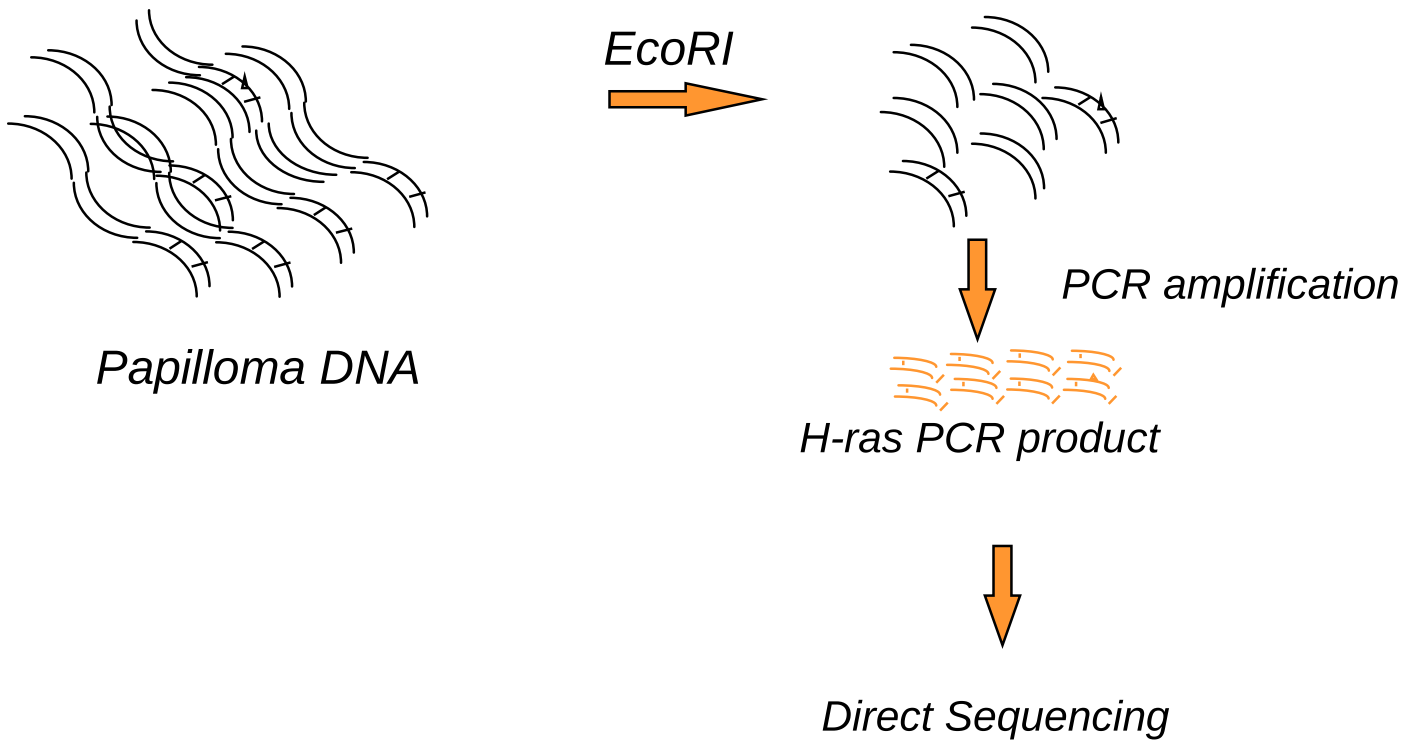


Fig. 1. Cell-cycle dependent initiation of hepatocarcinogenesis with BPDE. Groups of 15 rats each were treated with a single dose of BPDE at various times after a two-thirds partial hepatic resection (PHR). After 42 weeks of promotion with dietary phenobarbital, the mean yields of liver neoplasms were enumerated. Rats with intact livers were also treated with BPDE and promoted with phenobarbital (I).

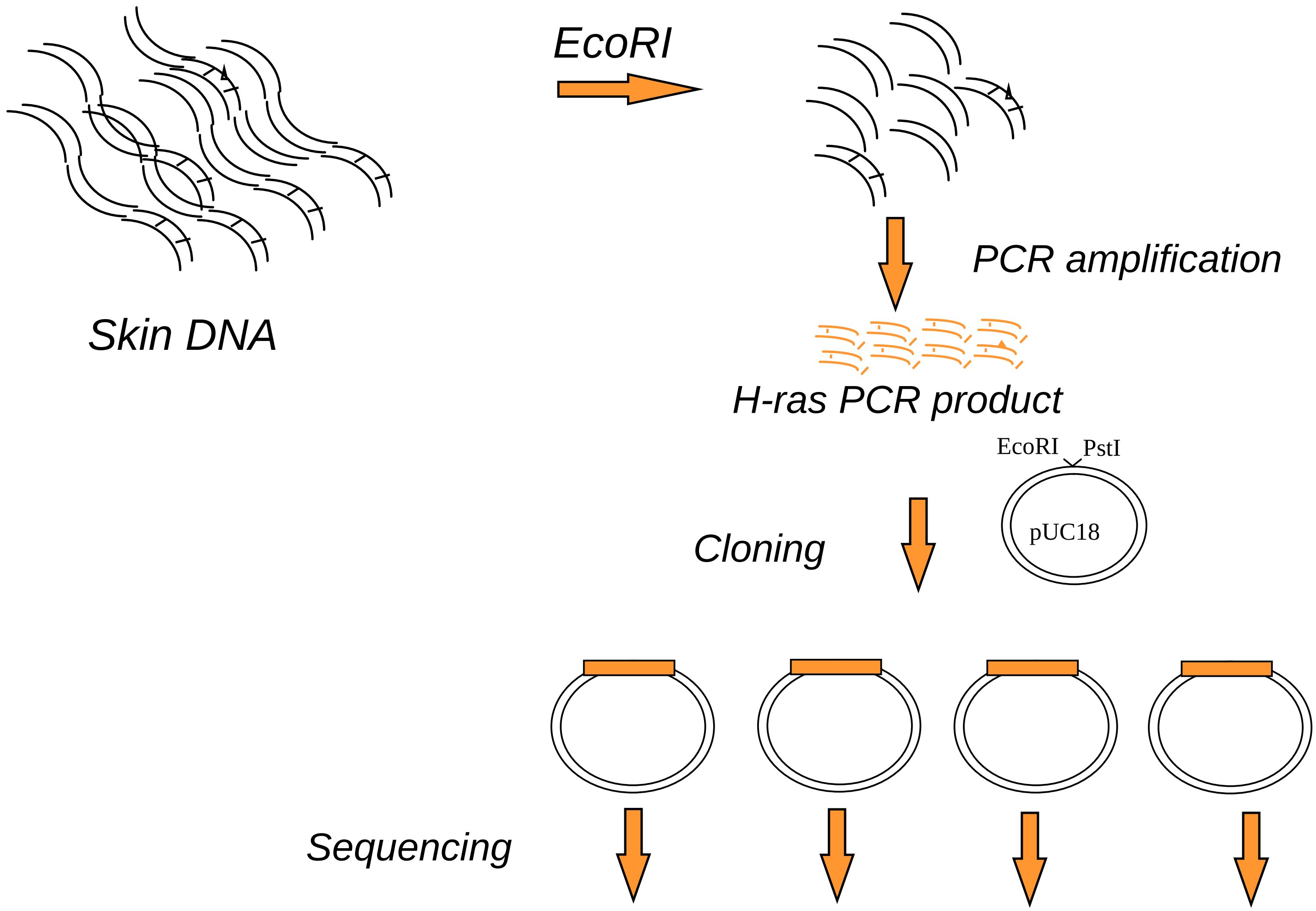


Kaufmann (1995) *Cancer Metastasis Rev*. 14: 31-41.
Moriya et al. (1996) *Biochemistry* 35: 16646-51
Perlow (2002) J Mol Biol 321:29-47

Papilloma mutations

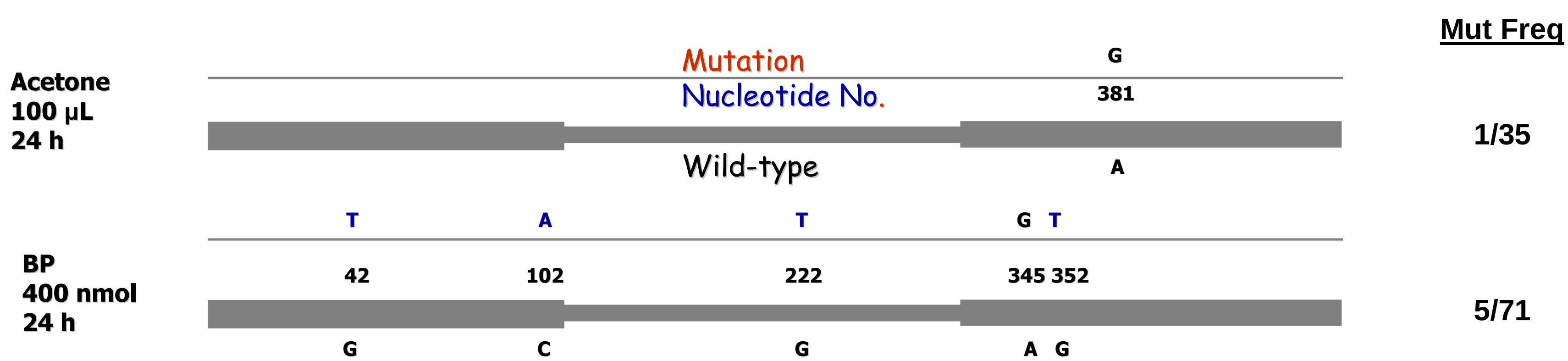


Preneoplastic mutations



Mutagenesis by BP

PAH	DNA adducts in mouse skin		H-ras mutations in papillomas		
	Stable adducts (% total)	Depurinating adducts (% total)	Codon	Sequence	No/Total papillomas
BP 400 nmol	G (23) Other (6%)	G (46%) A (25%)	13 61	GGC to GTC CAA to CTA	11/22 5/22



Chakravarti et al. (1995) PNAS 92: 10422
Chakravarti et al (2006) Submitted

Conclusions…

- BP-depurinating adducts show an excellent correlation with *ras* mutations in the papillomas.
  - Although the stable N^2-dG-BPDE adduct can form the codon 13 mutation, but no study showed the stable N^6-dA-BPDE adduct can form the codon 61 mutation.
- BP can form preneoplastic mutations in the pre-replication (repair) period.

On the other hand, DMBA forms more tumors from resting cells

One-time DMBA treatment

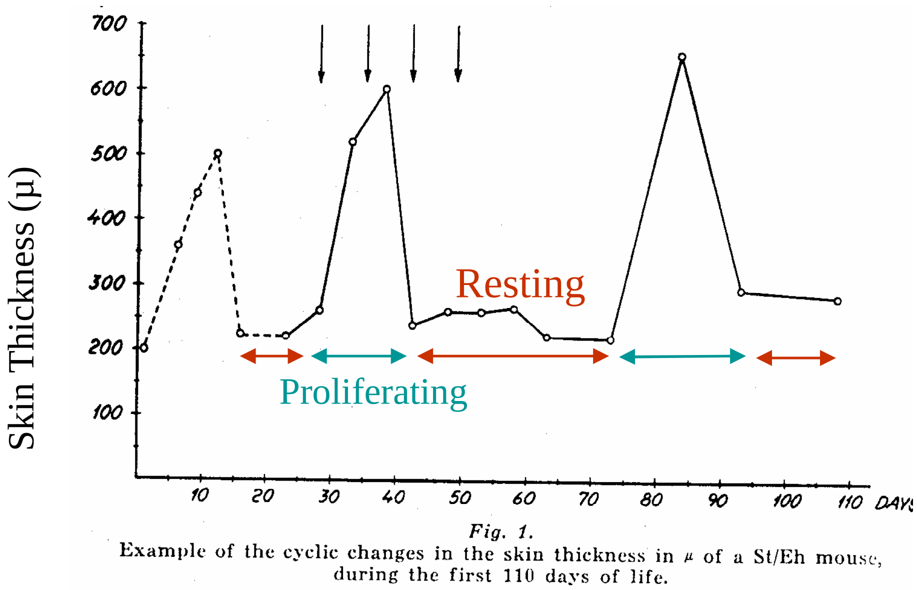
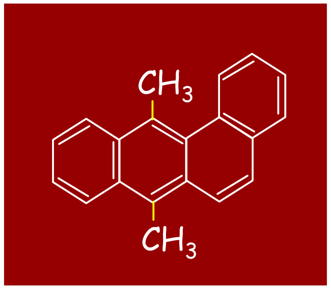


Fig. 1.
Example of the cyclic changes in the skin thickness in μ of a St/Eh mouse, during the first 110 days of life.

Age of mice (days)



7,12-dimethylbenz[*a*]anthracene (DMBA)

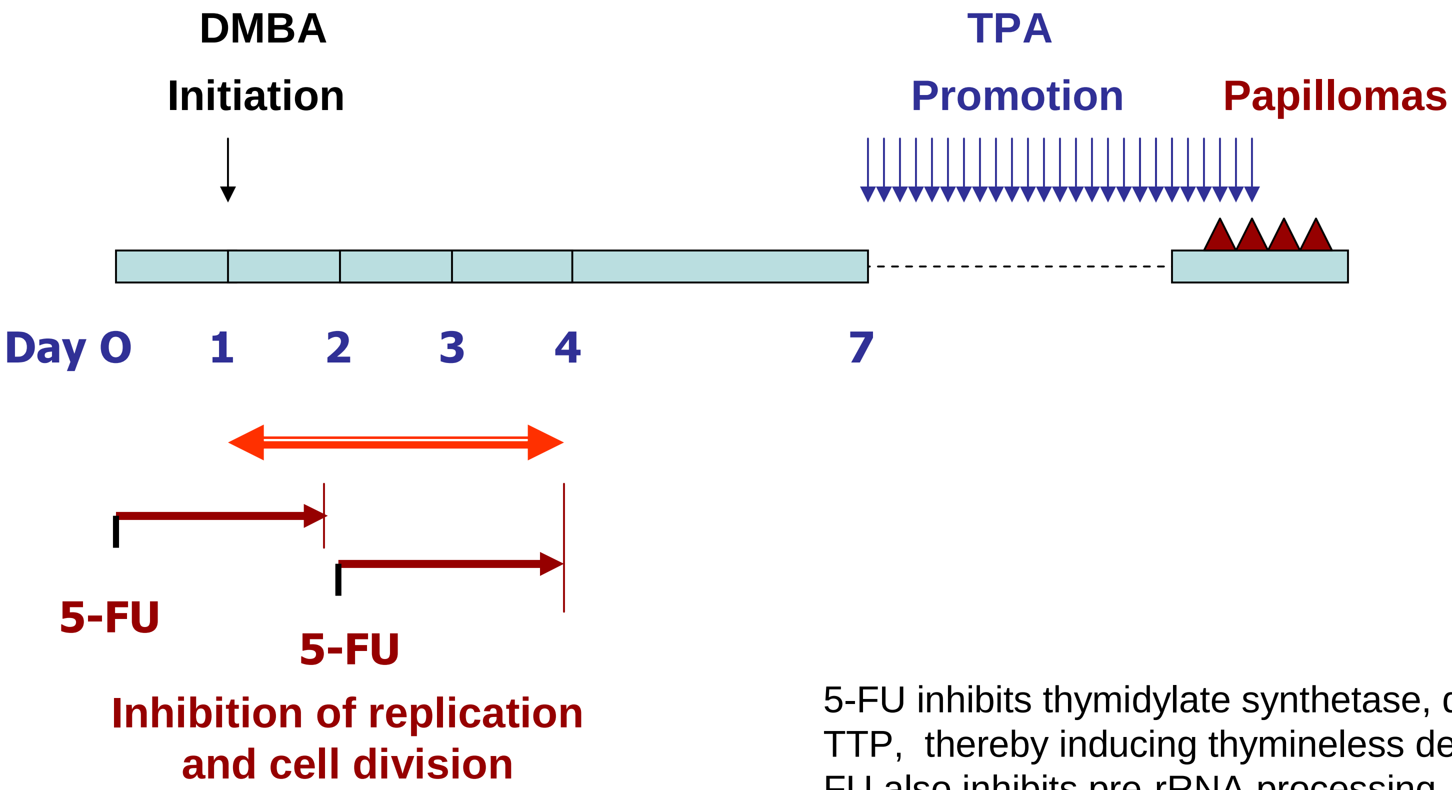
Skin cycle	Papillomas/Mice	
	Male	Female
Proliferating	1/30	5/26
Resting	15/34	22/34

Andreasen (1953) *Acta Path Scand* **32**: 165

Conclusions…

- Resting cells form more tumors than dividing cells
  - Results are against the idea that replication error over stable adducts is the major mechanism of generating the tumor-forming mutations

Blocking replication/cell division does not reduce DMBA tumor yield very much



5-FU inhibits thymidylate synthetase, depleting TTP, thereby inducing thymineless death. 5-FU also inhibits pre-rRNA processing.

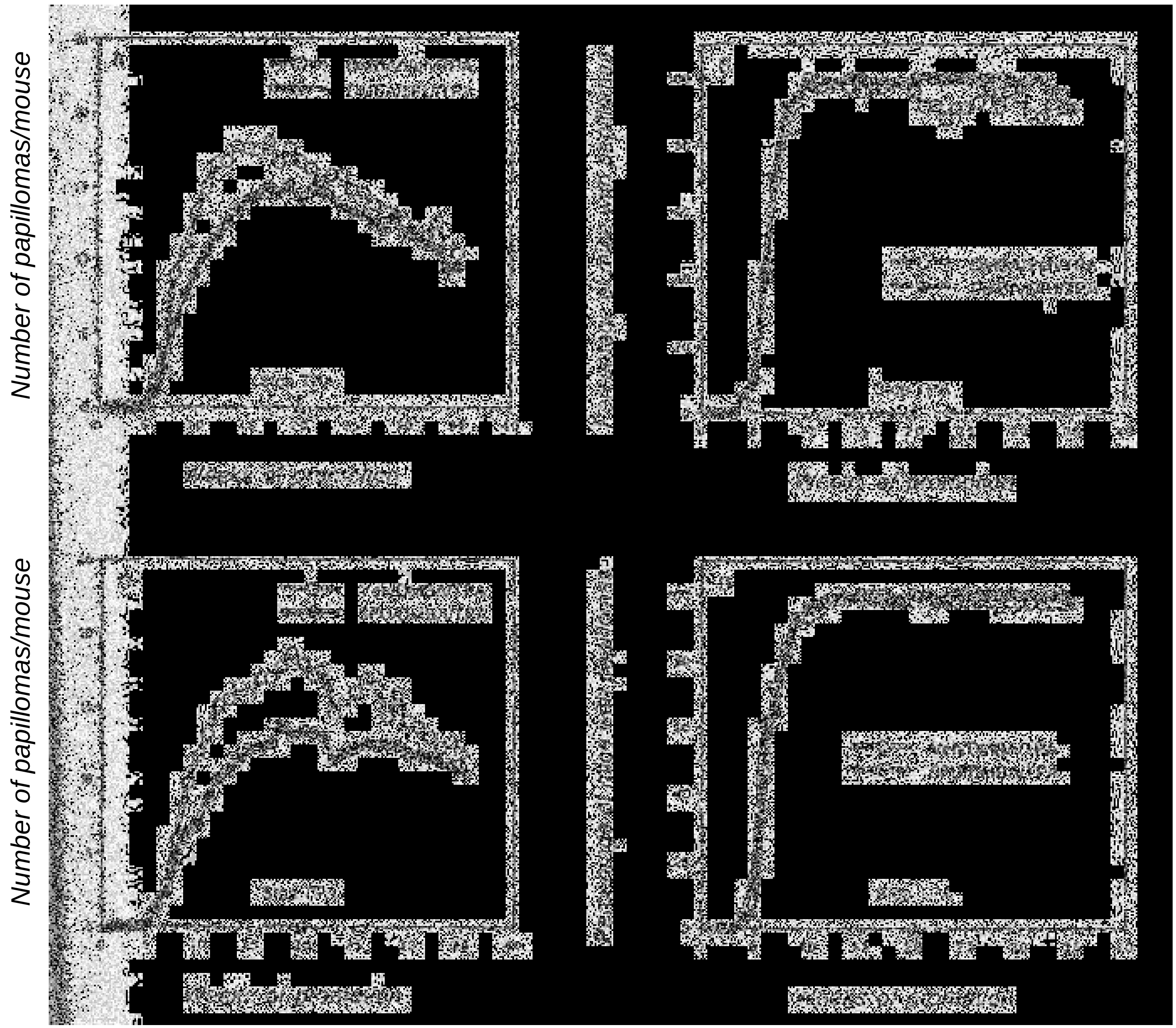
Ghosal and Jacob (1997) Biochem Pharmacol 53: 1569-75

Morris (1997) Cancer Res 57: 3436-43

5-FU

1d after DMBA treatment

1d before DMBA treatment



Conclusions…

- Replication/cell division in the first 4 days is not critical for DMBA tumor formation

  If the first 3 days have any role in forming the initiating mutations, they should be in the non-proliferating cells, and that mechanism should be refractory to 5-FU

  - DNA repair is not blocked by 5-FU

- Another study found that hydroxyurea treatment does not significantly reduce tumor yield by β-propiolactone [Smith et al (1968) Cancer Res 28: 2217-27]

Does DMBA form mutations in the first few days ?

H-*ras* codon 61 mutations in preneoplastic mouse skin

Nelson et al (1992) PNAS 89: 6398-402
Finch et al (1996) Carcinogenesis 17: 2551-7

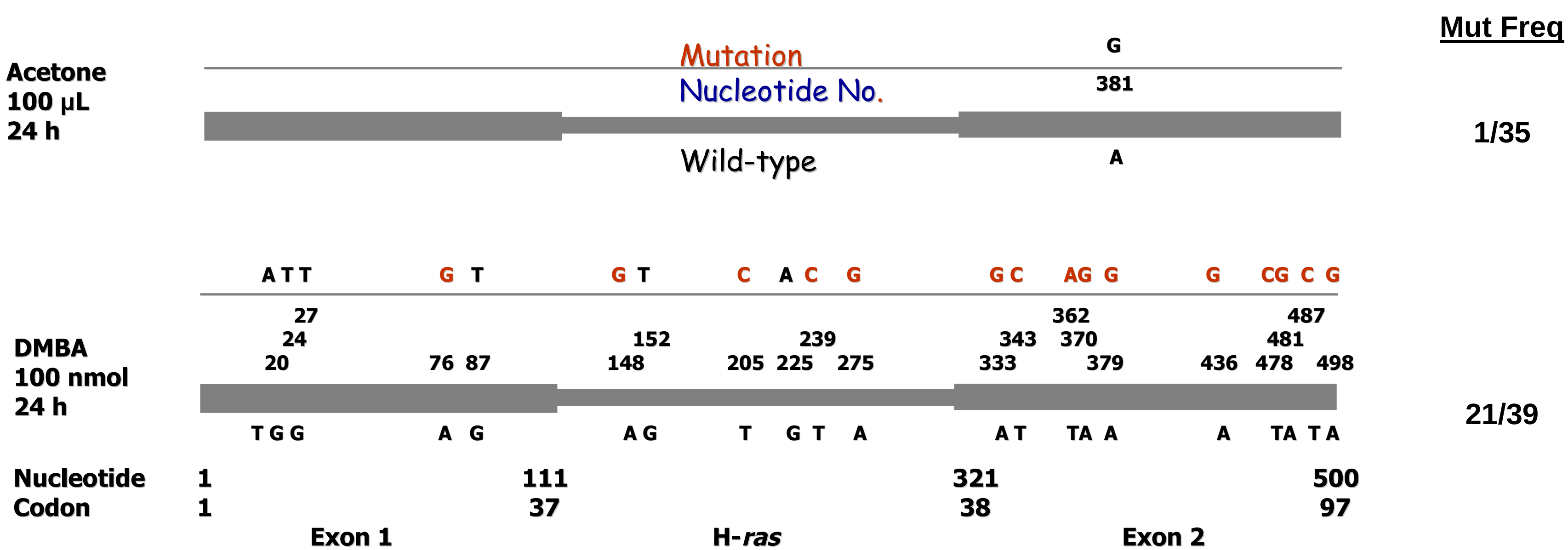
Conclusion…

- The first studies did not show that DMBA can form mutations in the pre-replication period

- However, we have observed mutagenesis in this time period

Mutagenesis by DMBA

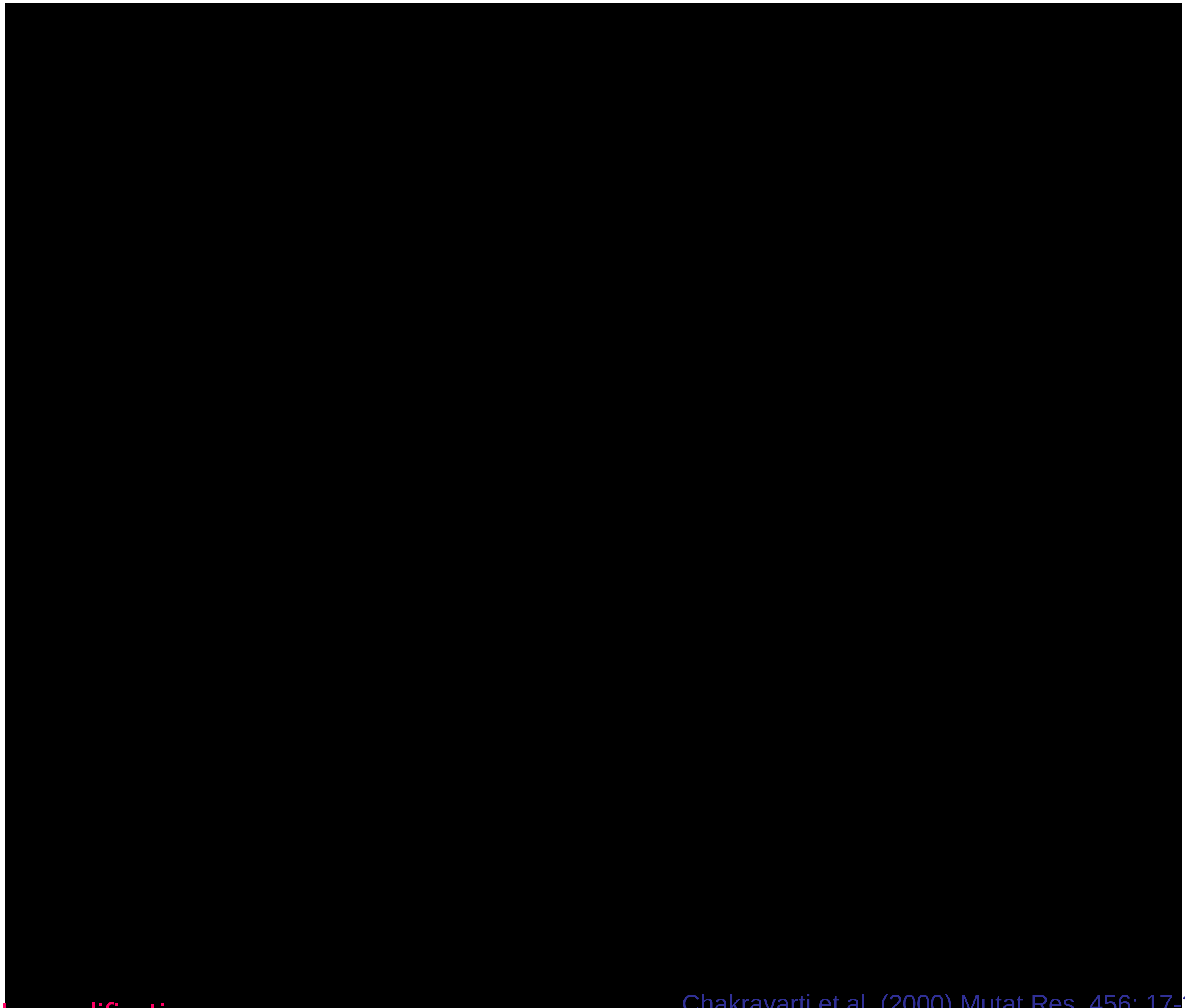
PAH	DNA adducts in mouse skin		H-ras mutations in papillomas		
	Stable adducts (% total)	Depurinating adducts (% total)	Codon	Sequence	No/Total papillomas
DMBA 100 nmol	Other (0.9%)	G (20%) A (79%)	13 61	GGC to GTC CAA to CTA	0/10 10/10



Chakravarti et al. (1995) PNAS 92: 10422
Chakravarti et al (2006) Submitted

Conclusions…

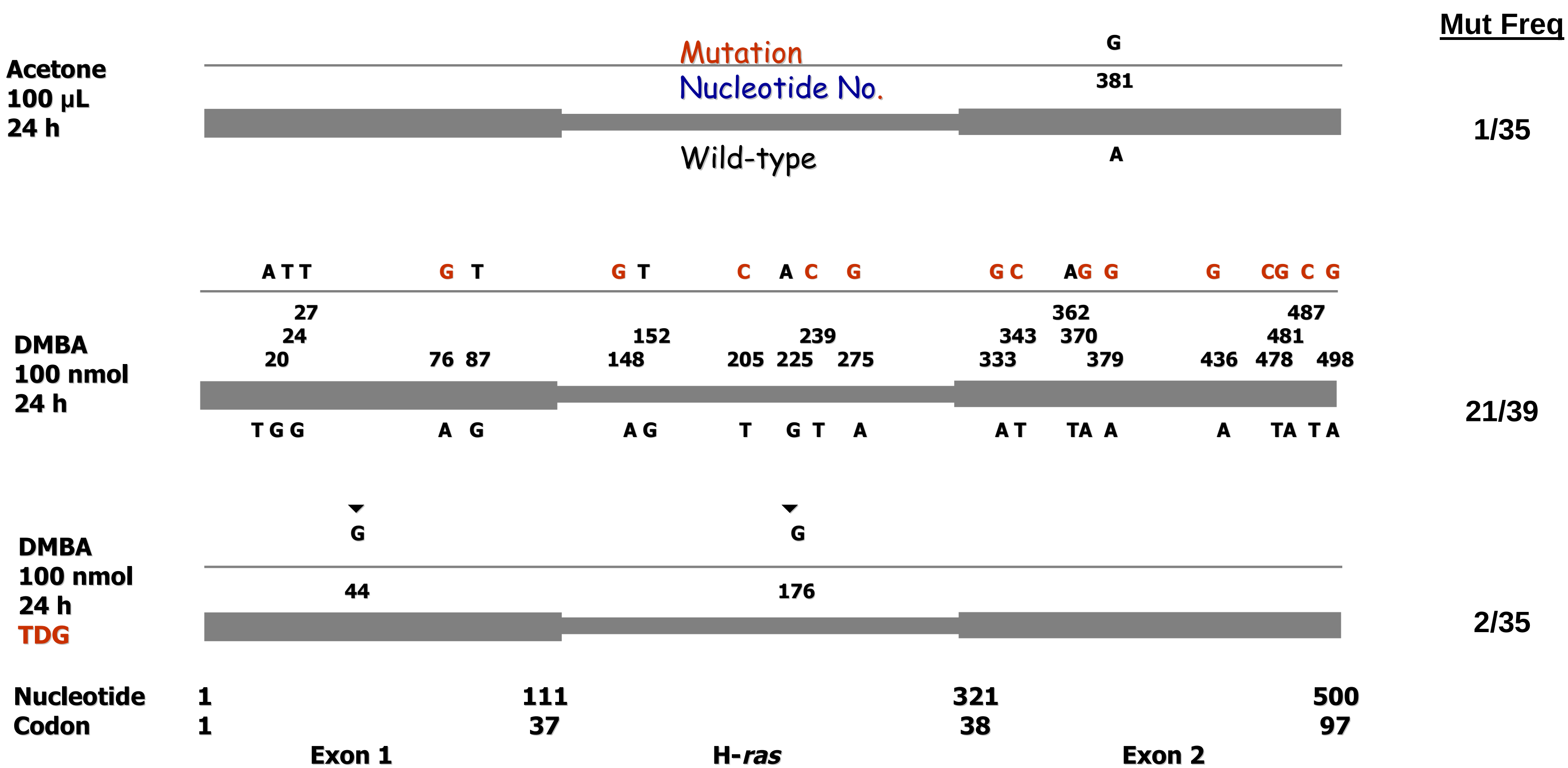
- DMBA-depurinating adducts show a strong correlation with ras mutation in the papillomas
- DMBA <u>does</u> induce mutations 1 d after treatment to mouse skin
- Since preneoplastic mutations are seen within the repair period, it is likely that they are formed by errors in repair.
  - If so, the A.T to G.C mutations should be induced either as G.T or A.C heteroduplexes



No amplification

Chakravarti et al. (2000) Mutat Res. 456: 17-32

DMBA forms G.T heteroduplex mutations



Chakravarti et al. (1995) PNAS 92: 10422
Chakravarti et al (2006) Submitted

Conclusions…

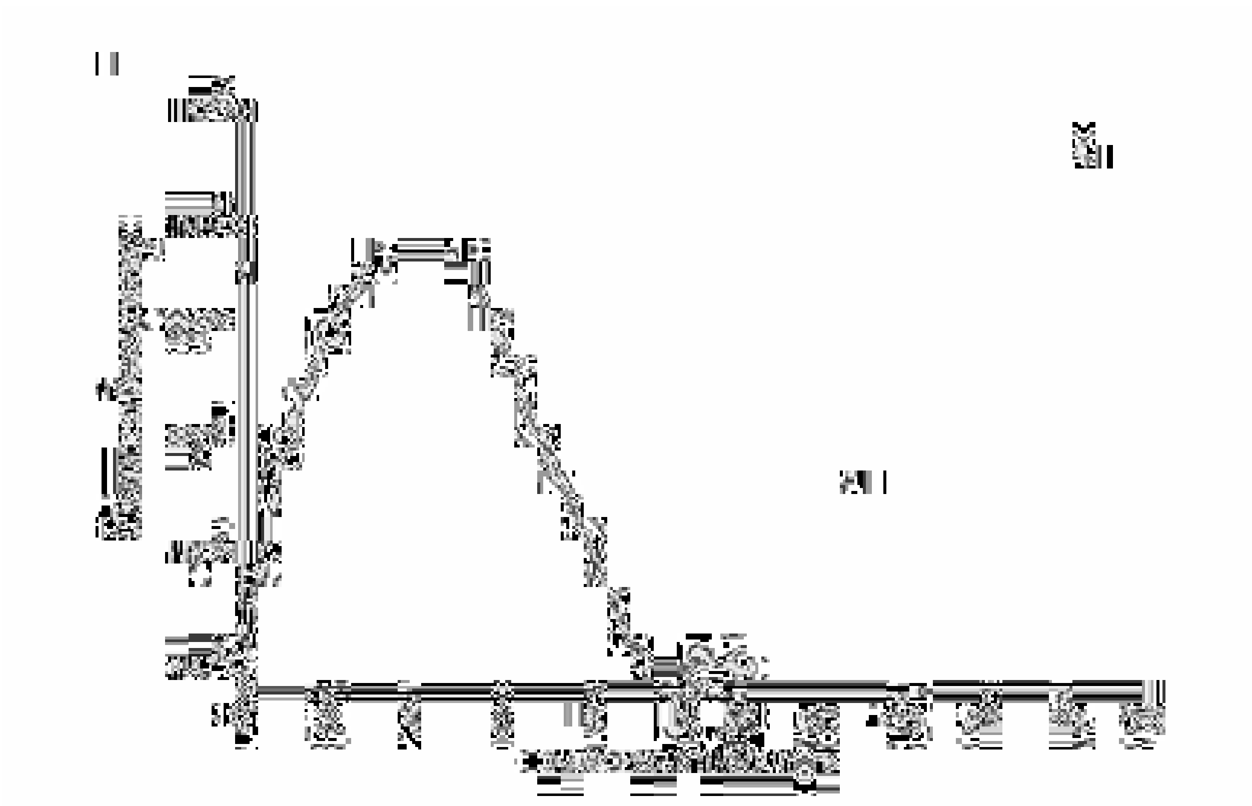
- Non-proliferating cells are the major source of DMBA tumors.
  - Errors in base excision repair of abasic sites formed by DMBA generate the necessary *ras* mutations.

Dibenzo[*a,l*]pyrene

- In truth, we first discovered the role of BER errors in generating tumor-forming mutations by studying DB[*a,l*]P.
  - Like DMBA, DB[*a,l*]P also forms 99% depurinating adducts, which form abundant amounts of abasic sites[1].
  - DB[*a,l*]P is a much stronger carcinogen than DMBA, and it was easy to detect the codon 61 mutations [2].
  - DB[*a,l*]P also formed similar A.T to G.C mutations [3].

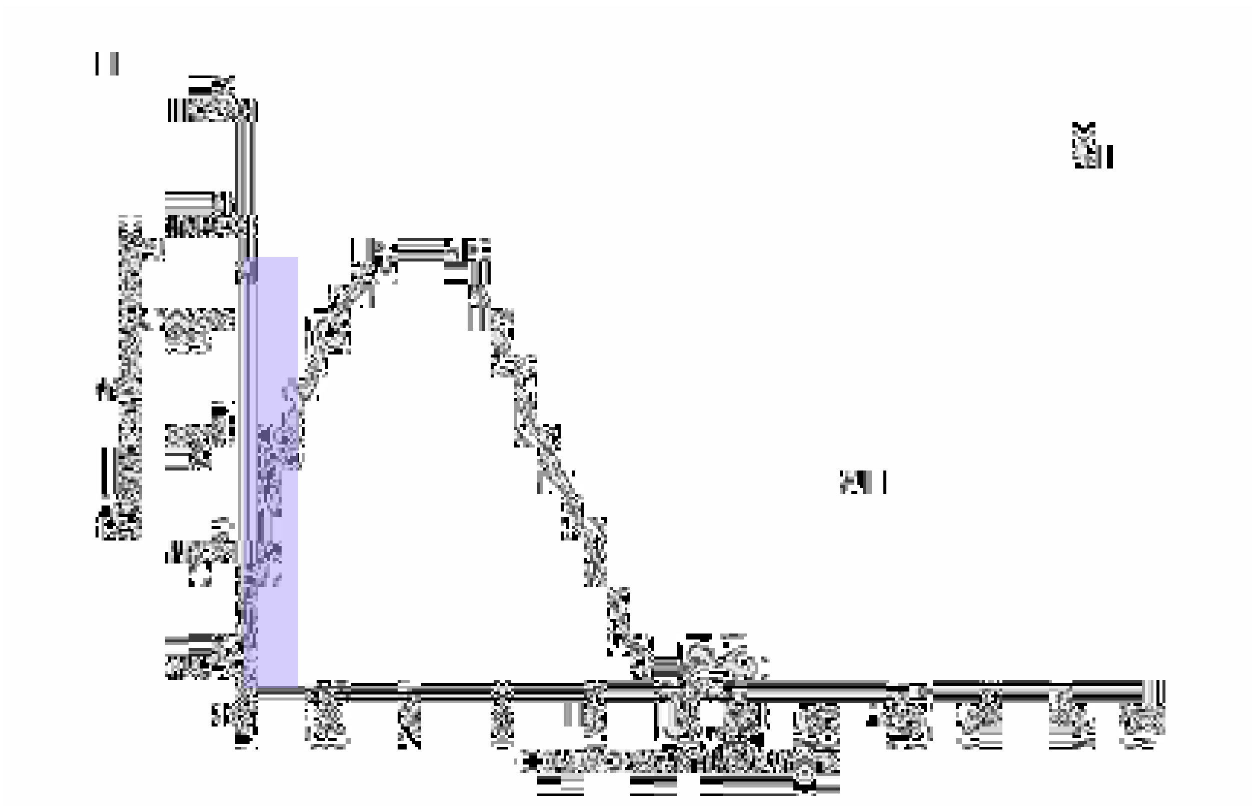
1. Chakravarti et al (2005) Mutat Res 588: 158
2. Chakravarti et al (1998) Oncogene 16: 3203
3. Chakravarti et al (2000) Mutat Res 456: 17
4. Chakravarti et al (2006) Submitted

Formation of H-*ras* codon 61 (CAA to CTA) mutations in DB[*a,l*]P-treated mouse skin



Chakravarti et al. (1998) Oncogene 16: 3203

Formation of H-*ras* codon 61 (CAA to CTA) mutations in DB[*a,l*]P-treated mouse skin



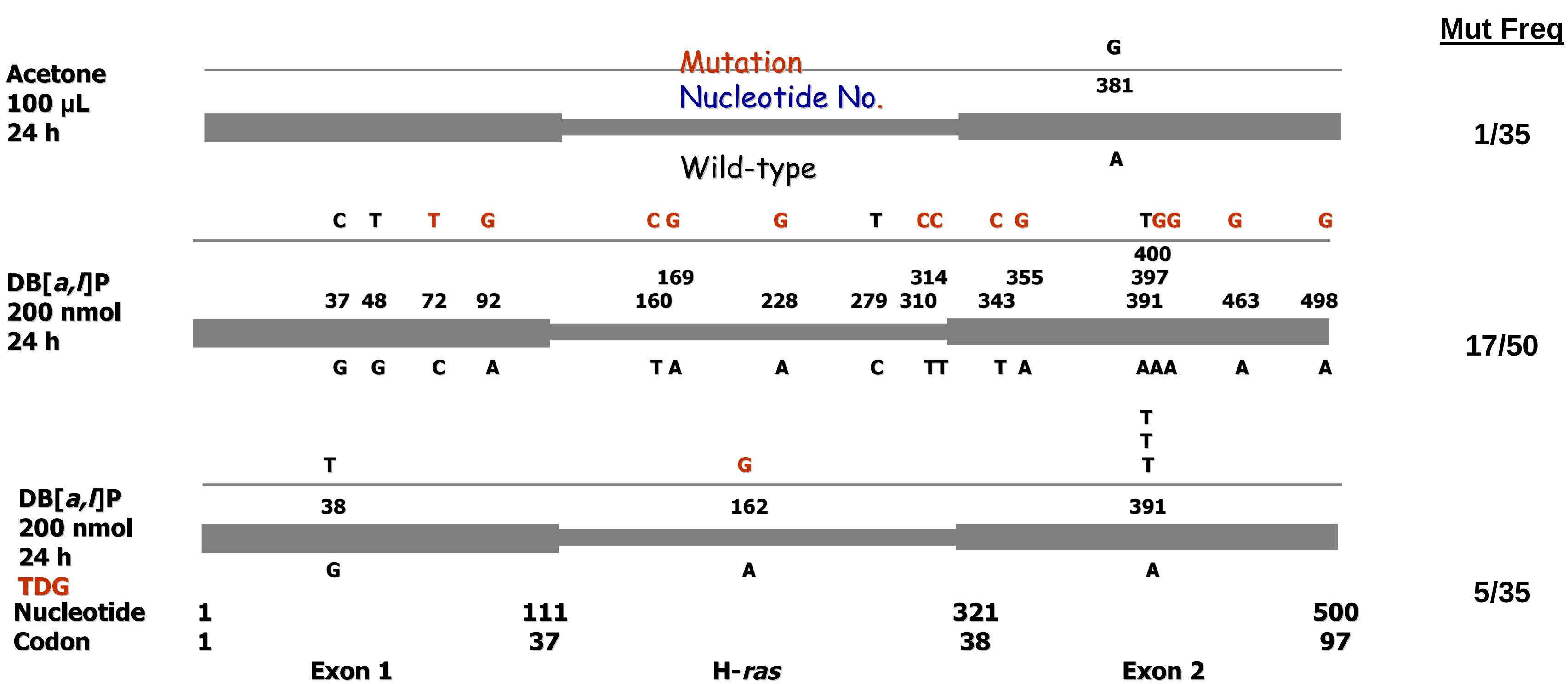
Chakravarti et al. (1998) Oncogene 16: 3203

Conclusions…

- DB[*a,l*]P forms the codon 61 mutations in the pre-replication repair period

Mutagenesis by DB[*a,l*]P

PAH	DNA adducts in mouse skin		H-ras mutations in papillomas		
	Stable adducts (% total)	Depurinating adducts (% total)	Codon	Sequence	No/Total papillomas
DB[*a,l*]P 200 nmol	G (0.5%) A (0.5%)	G (18%) A (81%)	13 61	GGC to GTC CAA to CTA	0/12 11/12



Chakravarti et al. (1995) PNAS 92: 10422
Chakravarti et al (2006) Submitted

Conclusions…

- Like DMBA, DB[*a,l*]P shows a strong correlation between depurinating adducts and ras mutations in the papillomas

- Like DMBA, DB[*a,l*]P shows a strong correlation between depurinating adducts and preneoplastic mutations
  - A.T to G.C mutations were most common, and they were formed as G.T heteroduplexes

The Estrogen story

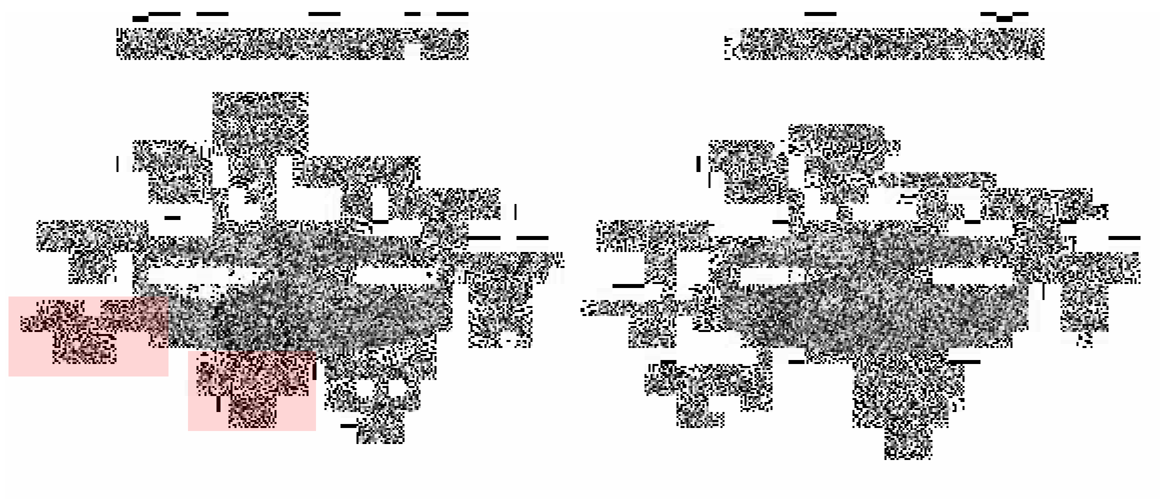
- Estrogen is a weak carcinogen. It was previously considered non-genotoxic due to negative Ames tests [1,2]

- However…

1. Dhillon (1995) Mutat Res 345: 87
2. Cavalieri and Rogan, Unpublished results

Estrogens and Breast cancer

- BRCA1-deficient women are particularly susceptible to breast cancer from estrogen exposure during pregnancy



P53 mutations in cancer (From the IARC database)					
Exon	Codon		Germline	Breast	Lung
5	133	A.T to G.C	7%	0.1%	1%
	163	A.T to G.C	0.2%	2.55%	0.67%
	179	A.T to G.C	-	2.2%	1.34%
	181	G.C to A.T	2.1%	0.46%	-
6	220	A.T to G.C	1.8%	3.5%	0.84%
7	235	A.T to G.C	2%	1%	0.3%
10	337	G.C to A.T	5.6%	0.2%	0.5%

Summary…

In both hereditary and sporadic breast cancers, estrogens may be associated with the induction of A.T to G.C mutations in the p53 gene

Estradiol-3,4-Quinone forms mainly A.T to G.C mutations

Animal	Treatment	Mutations After 6-24h	Frequency	
			Mutation/No.of clones	Mutations/Total Mutations
SENCAR mouse skin	Untreated	A.T>G.C	1/36(3%)	1/1
	E_2-3,4-Q	A.T>G.C	9/59 (15%)	9/13 (69%)
	+ TDG	A.T>G.C	0/74	0/4
ACI rat mammary gland	Untreated	A.T>G.C	18/95 (19%)	18/24 (75%)
	E_2-3,4-Q	A.T>G.C	30/63 (48%)	30/39 (77%)
	+ TDG	A.T>G.C	16/79 (20%)	16/20 (80%)

Chakravarti et al. (2001) Oncogene 20: 7945
Mailander et al (2006) J Steroid Biochem Mol Biol 101: in ptess

Conclusions…

- In mouse skin and rat mammary gland, estrogen quinone formed predominantly A.T to G.C mutations. This type of mutations is found in breast cancer
  - These mutations are induced as G.T heteroduplexes, as would be expected if these mutations are induced by erroneous BER of estrogen-induced abasic sites

So… what can we expect from Naphthalene ?

- Naphthalene quinone-DNA adducts may form mutations
  - What could be the mechanism?
    - Depurinating adducts should induce mutations by errors in BER
    - Stable adducts
      - Could induce mutations by errors in replication
      - Or, if the naphthalene-stable adducts are cleavable by AP endonuclease (as is the case with benzene [1]), erroneous BER would be the mechanism of mutagenesis.
  - Adducts and their Relevance to cancer
    - Our studies with PAH strongly indicate that depurinating adducts can form the *ras* mutations that establish the tumors
    - On the other hand, the role of stable adducts in forming oncogenic *ras* mutations is less certain.

1. Singer et al (1997) Chem Res Toxicol, 10: 713

Acknowledgements

Lab Members

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